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By VICTOR JULIUS TULANE

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
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STUDIES IN THE SYNTHESIS OF HIPPURIC ACID IN THE ANIMAL ORGANISM

VIII. HYDRAZINE INTOXICATION AND HIPPURIC ACID SYNTHESIS IN THE RABBIT

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(Received for publication, August 11, 1933)

Despite the very considerable amount of experimental work which has been concerned with the problem of hippuric acid synthesis, the evidence as to the organ or organs which function importantly in this synthesis is conflicting. Chief among the organs which have been considered as active in the formation of hippuric acid are the kidneys and the liver. The literature has been well summarized by Quick (1). While the hypothesis that, in the dog, the synthesis of hippuric acid occurs only in the kidney appears to be well founded (1), the site of synthesis in other laboratory animals such as the rabbit has not been extensively studied. It is notable that the rabbit and the dog differ significantly in their response to the administration of benzoic acid or its salts.

The investigations to be reported in the present communication are concerned with the rate of synthesis of hippuric acid in normal rabbits and in rabbits poisoned by the injection of hydrazine. Hydrazine was chosen because it is believed to be a toxic agent which affects primarily the liver (2, 3). Since it has been maintained that liver damage results in a depression of the synthesis of hippuric acid (4, 5), it was hoped that studies of hydrazine poisoning might offer some evidence as to the rôle of the liver in the detoxication of benzoic acid in this species.

As far as we have been able to ascertain, experimental work con-

* This work was done during the tenure of a scholarship from Howard University, made available by a grant from the General Education Board,

cerned with the influence of hydrazine on hippuric acid synthesis has been limited to the studies of Lackner, Levinson, and Morse (5) on dogs. These experiments are of doubtful significance, since the analytical methods used were not adequate and failed to distinguish between benzoic acid, free or in combination either with glycine or glucuronic acid. It was considered that all the benzoic acid present was conjugated with glycine as hippuric acid.

EXPERIMENTAL

The studies were carried out with male rabbits ranging in weight from 1.9 to 3.4 kilos, which were maintained on a uniform daily diet of 125 gm. of cabbage and 70 gm. of oats. On the experimental days on which benzoic or hippuric acid was administered, food was withheld for 24 hours. The animals were kept in the usual metabolism cages and the urines were collected quantitatively over 6, 18, or 24 hour periods. Any urine voided in the cage was received into a bottle containing 2 per cent nitric acid in order to prevent any hydrolysis of hippuric acid which might occur in an alkaline urine on standing (6).

The benzoic acid and hippuric acid were fed as the sodium salts through a soft rubber catheter introduced into the stomach in amounts equivalent to 660 mg. of benzoic acid per kilo of body weight. It has been suggested that in impairment of the function of the liver, defective hippuric synthesis may be occasioned by the failure of the liver to supply glycine in amounts adequate for conjugation with the benzoic acid (7, 8). It seemed particularly important, therefore, in the present series of experiments to provide an adequate supply of glycine, so that if any changes in the hippuric acid elimination were observed in hydrazine poisoning, they might be ascribed to a delayed or faulty synthesis of the hippuric acid and not to a lack of glycine for synthesis. Since Griffith and Lewis (9) showed that the elimination of hippuric acid by the rabbit was more rapid if glycine were administered with the benzoic acid, we have fed glycine in these experiments in amounts equivalent to 3 mols of glycine for each mol of benzoic acid fed.

The general procedure consisted in the determination of the rate of excretion of hippuric acid by the normal animal after the ingestion of benzoic acid or hippuric acid and glycine. This was

usually repeated several times in order to determine the normal variation in the rate of the synthesis and excretion in each animal. The experiments were then repeated after the production of hepatic injury by the subcutaneous injection of hydrazine sulfate (usually 87 mg. per kilo of body weight). The most marked symptoms of intoxication with this dosage were evident within 6 hours. The benzoic (or hippuric) acid and glycine were fed 6 hours after the injection of the hydrazine. After 24 hours, the toxic symptoms of hydrazine poisoning were rarely to be observed and, by the 2nd day, the animals usually ate the daily ration readily. At the end of the experiments, the animals were killed and pathological examination of the livers was made possible by the co-operation of Dr. Carl V. Weller of the Department of Pathology of this University. The typical pathology of the liver poisoned with hydrazine (2, 3) was almost invariably seen.

Free benzoic acid was determined by the method of Raiziss and Dubin (10), benzoic acid in conjugation with glycine (*i.e.*, hippuric acid) by the method of Griffith (11), and total benzoic acid by the method of Kingsbury and Swanson (12). As a check on the completeness of the collection of the samples, particularly in the 6 hour periods, creatinine was determined by the usual colorimetric method.

DISCUSSION

All the figures in Tables I to V are calculated in terms of "extra" benzoic acid; *i.e.*, the amount of benzoic acid excreted in the urine of the experimental period minus the normal excretion of benzoic acid for the period under consideration (as determined by analysis of the urines of the normal control periods in which no benzoic acid was fed). All the percentages represent the excretion of "extra" benzoic acid calculated as percentage of the benzoic acid fed. We consider the values for the 6 hour periods immediately following administration of the acid as most significant, since it has been shown (9), that, if an abundant supply of glycine is available, rabbits excrete the greater part of the ingested benzoic acid as hippuric acid within this period. Moreover, it was believed that, since hydrazine in non-lethal doses could hardly be expected to produce a complete lack of function of the liver within 6 hours, variations in the rate of excretion over the

shorter period would be far more significant as an indication of impairment of liver function than the total synthesis and excretion in the longer period of 24 hours. This expectation was borne out by the results of the experiments, since, in hydrazine poisoning, the total excretion of hippuric acid was seldom significantly altered in the 24 hour period, while the amount of hippuric acid synthesized and excreted in the first 6 hour period showed notable decreases.

TABLE I

Excretion of "Extra" Benzoic Acid by Normal and Hydrazinized Rabbits after Oral Administration of Sodium Benzoate and Glycine

The results are calculated as per cent of ingested benzoate eliminated as total benzoic acid and as hippuric acid.

Period	Extra benzoic acid excretion					
	Total			As hippuric acid		
	Maximum	Minimum	Average	Maximum	Minimum	Average
<i>hrs.</i>						
Normal (37)*						
6	91.0	54.0	71.0	81.0	48.9	65.3
18	41.0	9.0	24.0	41.0	8.0	22.0
24			95.0			87.3
Hydrazine poisoning (6)*						
6	58.0	34.4	46.7	58.0	31.2	43.7
18	46.2	23.5	40.0	44.9	32.2	38.7
24			86.7			82.4

* Figures in parentheses refer to the number of experiments included in the average.

Inspection of Table I, in which the results of all the experiments with sodium benzoate are summarized, shows that in normal rabbits during the first 6 hour period after the administration of the benzoate, approximately two-thirds of the ingested benzoic acid was excreted as hippuric acid and about 87 per cent in the 24 hour period, figures which are in agreement with those previously obtained (9) in this laboratory under similar conditions. After poisoning with hydrazine, the excretion for the 6 hour period was

much decreased. The average excretion in hydrazinized animals was 43 per cent of the intake during this period. On the other hand, the total excretion of hippuric acid in 24 hours, while slightly less than the normal value, was not significantly altered. That the decreased excretion of hippuric acid was not accompanied by an increased excretion of either free benzoic acid¹ or benzoic acid

TABLE II

Excretion of "Extra" Benzoic Acid in 6 Hour Period following Oral Administration of Sodium Benzoate and Glycine to Normal and to Hydrazinized Rabbits

The benzoic acid (660 mg. per kilo of body weight) was administered in the form of the sodium salt with 3 equivalents of glycine. Hydrazine sulfate (equivalent to 87 mg. per kilo of body weight except where indicated) was injected subcutaneously.

Rabbit No.	Benzoic acid fed	Normal				After hydrazine			
		Extra benzoic acid excreted							
		Total			As hippuric acid		Total		As hippuric acid
	gm.	gm.	per cent intake	gm.	per cent intake	gm.	per cent intake	gm.	per cent intake
4	1.386	1.270	91.0	1.130	81.0				
	1.386	1.210	86.0	1.060	76.0				
	1.386	1.154	83.0	1.000	76.0	0.762	55.0	0.717	50.9
9*	1.786	1.170	65.0	1.150	64.0				
	1.786	1.420	79.0	1.325	74.0				
	1.786	1.489	83.5	1.381	78.0	0.787	44.1	0.719	40.3
13	1.786	1.204	67.5	1.148	64.4				
	2.244	1.288	57.4	1.206	53.8				
	2.244	1.505	66.6	1.344	59.9	0.773	34.4	0.700	31.2

* Hydrazine sulfate equivalent to 65 mg. per kilo of body weight was injected subcutaneously.

in conjugation with some substance other than glycine is evident from a consideration of the relation between the values for total

¹ It may be noted that the excretion of free (unconjugated) benzoic acid has been shown to be very slight if glycine is fed with the benzoate and the amount of benzoate administered is moderate (9). No significant amounts of free benzoic acid were excreted by either the normal or hydrazinized rabbits. The difference between the total benzoic acid and benzoic acid as hippuric acid represents, for the most part, benzoic acid conjugated with some substance other than glycine.

benzoic acid and for benzoic acid excreted as hippuric acid in the normal and hydrazinized animals. If the excretions for the 24 hour period alone were considered, there would be little indication of any abnormality in the conjugation of benzoic acid and the excretion of hippuric acid in hydrazine poisoning. Studies of the excretion over shorter periods of time show that, after the administration of hydrazine, the animals were unable to accomplish the synthesis and excretion as rapidly as did the normal rabbits.

TABLE III

Excretion of "Extra" Benzoic Acid by Normal and Hydrazinized Rabbits after Oral Administration of Sodium Hippurate and Glycine

The results are calculated as per cent of ingested benzoate eliminated as total benzoic and as hippuric acid.

Period	Extra benzoic acid excretion					
	Total			As hippuric acid		
	Maximum	Minimum	Average	Maximum	Minimum	Average
<i>hrs.</i>						
Normal (14)*						
6	80.0	51.6	67.8	72.2	49.9	62.6
18	51.6	16.0	29.0	43.4	16.0	27.1
24			96.8			89.7
Hydrazine poisoning (4)*						
6	46.5	16.9	30.9	43.0	15.4	28.4
18	63.6	53.2	57.5	59.0	45.0	52.5
24			88.4			80.9

* Figures in parentheses refer to the number of experiments included in the average.

This is still more clearly shown by Table II in which the excretions of the 6 hour period are presented in detail for three of the six experimental animals.

It was considered of interest to study also the influence of hydrazine on the excretion of hippuric acid in rabbits after oral administration of hippuric acid and glycine. The rate of excretion of hippuric acid after feeding hippuric acid is similar to that observed after feeding benzoate (13) and the simultaneous adminis-

tration of glycine with the hippuric acid likewise results in a significant increase in the rate of excretion of hippuric acid. These facts led Griffith (13) to believe that hippuric acid is hydrolyzed in the intestine (14) and that the absorbed benzoic acid is resynthesized to hippuric acid by the organism. In Tables III and IV are presented data, comparable to those of Tables I and II, obtained after oral administration of hippuric acid and glycine. In con-

TABLE IV

Excretion of "Extra" Benzoic Acid in 6 Hour Period following Oral Administration of Sodium Hippurate and Glycine to Normal and to Hydrazinized Rabbits

The hippuric acid (equivalent to 660 mg. of benzoic acid per kilo of body weight) was administered in the form of the sodium salt with 3 equivalents of glycine. Hydrazine sulfate (equivalent to 87 mg. per kilo of body weight except where indicated) was injected subcutaneously.

Rabbit No.	Hippuric acid fed, calculated as benzoic acid	Normal				After hydrazine			
		Extra benzoic acid excreted							
		Total			As hippuric acid		Total		As hippuric acid
	gm.	gm.	per cent intake	gm.	per cent intake	gm.	per cent intake	gm.	per cent intake
4	1.386	1.018	73.0	0.951	69.0				
	1.386	0.934	67.0	0.893	64.0	0.645	46.5	0.597	43.0
9*	1.782	0.919	51.6	0.890	49.9				
	1.782	0.971	54.5	0.893	50.0	0.535	30.0	0.495	27.8
13	2.244	1.690	75.3	1.620	72.2				
	2.244	1.324	59.0	1.260	56.2				
	2.244	1.489	66.4	1.427	63.6				
	2.244	1.360	60.5	1.327	59.2	0.380	16.9	0.329	15.4

* Hydrazine sulfate equivalent to 65 mg. per kilo of body weight was injected subcutaneously.

firmation of the observations of Griffith (13), the excretions of hippuric acid in the 6 hour periods after the oral administration of hippuric acid and of benzoic acid were practically identical. In hydrazine poisoning the rate of excretion of hippuric acid after feeding hippuric acid was also much depressed.

It is possible that the lowered rate of excretion observed in hydrazine intoxication might have been the result of a change in the permeability of the intestinal mucosa, due to the toxic effects of

hydrazine, which would diminish the rate of absorption of the benzoate and thus alter the rate of synthesis. Hydrazine is not known to influence the intestinal permeability and it seems improbable that the absorption could have been so greatly decreased as to result in the marked changes in the excretion of hippuric acid which were observed.

It has been shown (9) that there is no delay in the excretion of hippuric acid once formed and present in the blood stream. The rate of excretion of hippuric acid is at least as rapid as the rate of its synthesis in the normal rabbit. If hydrazine caused an impairment of kidney function, it is possible that the rate of synthesis would exceed the rate of excretion by the kidneys so that results similar to those observed in these experiments would be obtained. There is no evidence to indicate such an action of hydrazine. Direct and convincing proof of the rôle of changes in absorption from the intestine or impairment of the function of the kidneys in influencing hippuric acid synthesis and excretion in hydrazine poisoning could have been obtained by studies, similar to those discussed here, in which sodium benzoate was introduced directly into the blood stream. Experiments of this type were attempted, but invariably the combined toxicity of the hydrazine and the benzoate was so great that the animals did not survive the injections.

Hydrazine is known to produce serious hepatic injury. Morgulis, Pratt, and Jahr (7) considered that the rôle of the liver in the conjugation of benzoic acid was a preliminary one; namely, the production of the glycine required. More recently Quick (8) has stated that the synthesis of glycine is effected by the liver and that, in hepatic disease, a decrease in the excretion of hippuric acid should be observed because of the inability of the liver to produce the glycine required for the synthesis. In the present work, the supply of glycine can hardly be a factor, since glycine in amount considerably in excess of that required for conjugation with the benzoic acid was fed. While we feel that the other factors discussed cannot be excluded entirely, we believe in the light of our own experiments and of other evidence that the decreased excretion of hippuric acid in hydrazinized rabbits is to be associated with hepatic injury and a failure to conjugate the benzoic acid with the glycine as effectively as in the normal animal.

Certain questions of interest concerning the ability of the organism of the normal rabbit to synthesize hippuric acid are suggested by these studies. As previously stated, all animals received the same amount of benzoic acid per kilo of body weight. However, owing to differences in the weights of the experimental animals, the absolute amounts of benzoic acid fed varied considerably. As the absolute amount of benzoic acid fed increased, there was not observed a proportionate increase in the excretion of hippuric acid in the 6 hour period. This is shown in Table V. Thus Rabbit 4, weighing 2.1 kilos, excreted in 6 hours 1.062 gm. of benzoic acid as hippuric acid or 76 per cent of the benzoic acid fed, while Rabbit 13, a much larger animal, weighing 3.4 kilos,

TABLE V

Excretion of "Extra" Benzoic Acid As Hippuric Acid in Rabbits of Varying Weight after Oral Administration of Sodium Benzoate and Glycine

All the animals received the same amount of benzoic acid per kilo of body weight, 0.660 gm.

Rabbit No.	Weight	Benzoic acid fed	Extra benzoic acid excreted as hippuric acid in 6 hr. period		No. of experiments averaged
			gm.	per cent intake	
4	2.1	1.386	1.062	76.6	3
9	2.7	1.782	1.251	70.2	4
7	3.2	2.112	1.216	57.3	7
13	3.4	2.244	1.207	53.8	3

excreted in the same period 1.207 gm. or 53 per cent of the amount fed. Quick (8) has made similar observations in man. Despite marked increases in the amount of benzoic acid fed, it was not possible to increase the hourly excretion of hippuric acid above a certain maximal value. He considered that this fixation of the excretion of hippuric acid in man was due to a limitation of the synthesis of glycine since the hourly excretion of hippuric acid could be increased markedly by the administration of glycine. In our experiments with rabbits, glycine equivalent to 3 times the amount of benzoic acid was fed with the benzoic acid, so that an excess of the amino acid over that needed for the synthesis should have been present, even when the larger amounts of benzoic acid were fed. The results suggest that there may be a maximal rate at which synthesis of hippuric acid can take place even in the presence of

an abundant supply of glycine. It is proposed to investigate this question further.

SUMMARY

1. A comparison has been made of the rate of excretion of hippuric acid after the oral administration of sodium benzoate and glycine to normal rabbits and to rabbits poisoned with hydrazine.

2. The excretion of hippuric acid during the first 6 hours after administration of the benzoate in hydrazine intoxication was much less than that of normal rabbits. Failure of a supply of glycine adequate for the synthesis was not a significant factor since 3 equivalents of glycine were always fed with the benzoate. The synthesis and excretion appeared merely to be delayed in hydrazine intoxication, since the total excretion over a period of 24 hours was only slightly less than that of normal animals.

3. The decreased excretion of hippuric acid was not accompanied by a correspondingly increased excretion of either free benzoic acid or benzoic acid conjugated with some substance other than glycine.

4. Similar results were obtained after feeding sodium hippurate and glycine to hydrazinized rabbits.

5. Since hydrazine is associated with hepatic injury primarily, the results of these experiments can best be explained on the basis of a delayed synthesis of hippuric acid by the liver, even in the presence of glycine in excess of the amount required for the combination with benzoic acid.

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STUDIES IN THE SYNTHESIS OF HIPPURIC ACID IN THE ANIMAL ORGANISM

IX. A COMPARATIVE STUDY OF THE RATE OF SYNTHESIS AND EXCRETION OF HIPPURIC AND PHENACETURIC ACIDS BY THE RABBIT

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(Received for publication, August 11, 1933)

The purpose of the experiments presented in this paper was to compare the behavior of benzoic acid and its next higher homologue, phenylacetic acid, in the organism of the rabbit. Phenylacetic acid, like benzoic acid, may be conjugated with glycine and the conjugation product, phenaceturic acid, the homologue of hippuric acid, excreted by the kidneys. Less attention has been given to the biological behavior of this acid than to that of the homologous benzoic acid. It is generally assumed that in the rabbit the site of synthesis of phenaceturic acid is the same as that of hippuric acid and that it is eliminated by the kidneys under the same conditions. During the progress of this work, Quick (1) reported a similar comparative study of the behavior of benzoic and phenylacetic acids in the dog. As will be shown, the rabbit and the dog differ somewhat in their response to the administration of these acids.

It is known that the organism of the rabbit can synthesize hippuric acid readily but that the supply of glycine available is an important factor which limits the *rate* of synthesis (2, 3) in this species. The rate of excretion of hippuric acid has been shown to be increased if glycine is fed with the benzoate. Is the synthesis of phenaceturic acid also readily accomplished in the organism of the rabbit? Can the formation and excretion of phenaceturic

* This work was done during the tenure of a scholarship from Howard University, made available by a grant from the General Education Board.

acid be facilitated by the administration of glycine with the phenylacetic acid? It is with these problems that the experimental work here reported is concerned.

EXPERIMENTAL

The procedure was similar to that used in earlier experiments on the rate of excretion of hippuric acid as influenced by the administration of glycine with the sodium benzoate (2) and to that of Paper VIII (4). Benzoic acid in amounts equivalent to 0.660 gm. per kilo of body weight was administered as the sodium salt and phenylacetic acid, likewise as the sodium salt, in amounts equivalent to 0.736 gm. per kilo (*i.e.*, the molar equivalent of 0.660 gm. of benzoic acid). In a few experiments, phenaceturic acid in amounts equivalent to the phenylacetic acid was administered. This phenaceturic acid, prepared from the urine of rabbits fed phenylacetic acid and glycine or gelatin by a slight modification of the method of Suzuki (5), melted at 142° and, on titration, a molecular weight of 195 was indicated (theoretical, 193). The sodium salts, when injected, were introduced into the marginal ear vein without the use of anesthetics.

As phenylacetic acid and its conjugated derivatives react in much the same manner as do benzoic acid and its conjugated derivatives, the same analytical methods were used for the determination of the total phenylacetic acid, free phenylacetic acid, and phenylacetic acid in combination with a nitrogenous compound (*i.e.* phenaceturic acid¹) as for the determination of the corresponding derivatives of benzoic acid, as outlined in Paper VIII (4). Since phenaceturic acid is less readily soluble in ether than is hippuric acid, the ether extraction in the Griffith method was continued for 2 hours in order to obtain complete removal of the acid by the ether.

DISCUSSION

The excretions during the first 6 hours immediately after the administration of the salts of the organic acids are represented

¹ Although glutamine is known to be conjugated with phenylacetic acid in man (6), so far as is known this mechanism of detoxication is not employed in the organism of the rabbit. Hence we have calculated all the material determined by the Griffith method after the administration of phenylacetic acid as phenaceturic acid.

graphically in Chart 1, which summarizes the experimental data obtained with five animals. The data obtained after the administration of benzoic acid with or without supplements of amino acids (glycine, alanine) are in harmony with the work previously reported from this laboratory (2) and differ markedly from the results of the experiments in which phenylacetic acid was adminis-

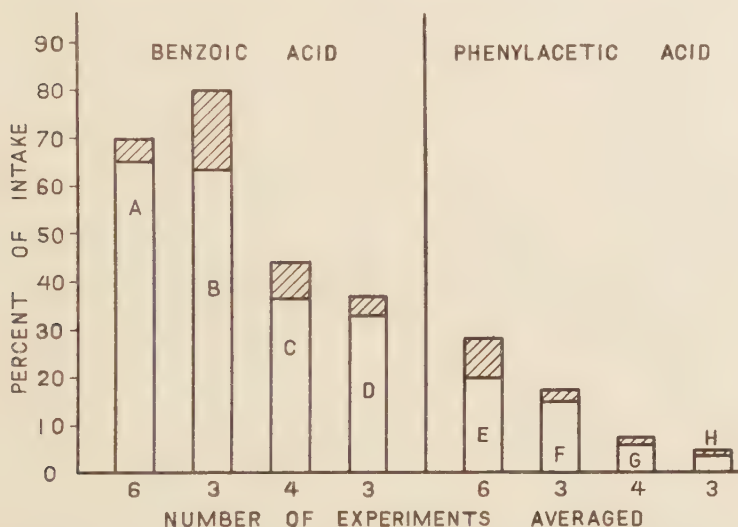


CHART 1. The average urinary excretion of "extra" benzoic and phenylacetic acids in the 6 hour period immediately after the administration of the sodium salts of these acids. The entire column represents the excretion of total benzoic or phenylacetic acid. The unshaded portion represents the amount of these acids excreted in conjugation with glycine (*i.e.* hippuric or phenaceturic acid). In series A, B, E, and F, 3 equivalents of glycine were fed; in series C and G, no amino acid was administered; and in series D and H, 3 equivalents of alanine were fed. The salts of the organic acids were administered orally except in series B and F, in which they were injected intravenously. All animals received equivalent amounts of the organic acids per kilo of body weight (see text).

tered. The elimination of hippuric acid was greatly increased when glycine was fed with the benzoate (A, Chart 1), while the feeding of alanine (D, Chart 1) did not result in an increased rate of excretion as compared with experiments in which no supplementary amino acid was fed with the benzoate (C, Chart 1).

That the effect of the glycine was not due to an increased rate of absorption of the benzoate from the intestine was shown by the experiments in which glycine was fed and the benzoate was injected directly into the blood stream (*B*, Chart 1). Although the total benzoic acid excreted was increased as compared with the excretion after the oral administration, the hippuric acid excretion was essentially the same. Since in previous work (2) hippuric acid when injected intravenously has been shown to be excreted rapidly, these results are interpreted as indicating that the rate of absorption of the benzoate from the intestine, in the dosage used, is at least as rapid as the rate of synthesis, even in the presence of glycine.

The excretion of phenaceturic acid occurred much less rapidly than did that of hippuric acid. When no glycine was fed with the phenylacetic acid (*G*, Chart 1), approximately 6 per cent of the amount administered was excreted as phenaceturic acid within 6 hours, as compared with approximately 37 per cent of the ingested benzoic acid as hippuric acid. The simultaneous administration of glycine with the phenylacetic acid (*E*, Chart 1), while it resulted in an increase in the excretion of phenaceturic acid, about 21 per cent of the ingested phenylacetic acid, did not result in as notable a synthesis of phenaceturic acid as was observed in the case of hippuric acid when the benzoate and glycine were fed together. The feeding of alanine did not influence the excretion of phenaceturic acid (*H*, Chart 1). Since the amount of phenaceturic acid in the urine in the 6 hour period was not greater when the phenylacetic acid was injected intravenously (*F*, Chart 1), slower absorption from the intestine can hardly be a factor of importance in the delay in conjugation of phenylacetic acid with glycine. Since phenaceturic acid, like hippuric acid, introduced directly into the blood stream (Rabbit 31, Table I) was found to be excreted rapidly by the kidneys, we believe that the experiments discussed indicate that the capacity of the organism of the rabbit to conjugate phenylacetic acid with glycine is inferior to its capacity to synthesize hippuric acid under comparable conditions. As is shown in Chart 1 (*E* and *G*), the supply of glycine was one factor which limited the synthesis of phenaceturic acid. Nevertheless, even in the presence of glycine, the rate of synthesis and excretion was much lower than the rate observed when benzoic acid was fed without any supplement of glycine.

TABLE I

Excretion of Free Benzoic, "Extra" Total Benzoic, and Hippuric Acids and Free Phenylacetic, "Extra" Total Phenylacetic, and Phenaceturic Acids in 6 Hour Period Immediately following Ingestion of Sodium Salt of Benzoic or Phenylacetic Acid Alone and with Glycine or Alanine

The amounts of the acids were molar equivalents, 0.660 gm. and 0.735 gm. of benzoic acid and phenylacetic acid per kilo of body weight. Except where indicated all the substances were administered orally.

Rabbit No.	Free benzoic or phenylacetic acid excreted		Extra benzoic or phenylacetic acid excreted as		Amino acid fed		Acid administered		
			Total	Hippuric or phenaceturic acid					
	gm.	gm.	per cent in-take	gm.	per cent in-take	mols	gm.		
14	0.007	1.405	85.2	1.273	77.2	Glycine 3	Benzoic	1.650	
	0.010	0.856	51.9	0.670	40.6	0	"	1.650	
	0.108	1.610	97.6	1.228	74.4	" 3	" *	1.695	
	0.010	0.662	33.4	0.562	28.4	Alanine 3	Benzoic	1.980†	
	0.107	0.606	33.0	0.507	27.6	Glycine 3	Phenylacetic	1.839	
	0.005	0.543	29.5	0.536	29.1	" 5	"	1.839	
	0.007	0.183	10.0	0.172	9.3	0	"	1.839	
	0.028	0.444	24.6	0.464	25.8	" 3	" *	1.800	
	0.040	0.095	4.3	0.085	3.9	Alanine 3	Phenylacetic	2.207	
29	0.007	1.182	74.5	1.129	71.3	Glycine 3	Benzoic	1.584	
	0.022	0.771	48.7	0.666	42.0	0	"	1.584	
	0.103	1.367	89.6	1.140	74.8	" 3	" *	1.525	
	0.139	0.606	39.7	0.367	24.0	0	" *	1.525	
	0.012	0.604	38.1	0.581	36.6	Alanine 3	Benzoic	1.584	
	0.005	0.356	20.2	0.341	19.3	Glycine 3	Phenylacetic	1.765	
	0.054	0.158	9.0	0.086	4.9	0	"	1.765	
	0.015	0.203	10.2	0.239	11.4	" 3	" *	2.000	
	0.070	0.114	6.5	0.101	5.7	Alanine 3	Phenylacetic	1.765	
	0.010	0.514	29.1	0.505	28.6	0	Phenaceturic	2.506	
	31	0.033	0.822	65.5	0.800	63.8	Glycine 3	Benzoic	1.254
		0.005	1.024	64.6	0.984	62.1	" 3	"	1.584
0.006		0.668	42.2	0.606	38.2	Alanine 3	"	1.584	
0.006		0.746	47.1	0.627	39.6	0	"	1.584	
		0.388	21.9	0.360	20.4	Glycine 3	Phenylacetic	1.765	
0.006		0.069	4.0	0.065	3.7	Alanine 3	"	1.765	
0.070		0.126	7.1	0.106	6.0	0	"	1.765	
		1.348	89.3	1.331	88.1	0	Phenaceturic*	1.510	

* Intravenous administration.

† These experiments were carried out at intervals of a week or more between administrations of the organic acids. Since the animals sometimes increased in weight, this explains the increase in the absolute amount of acid. The dose per kilo remained the same.

These facts and certain others of interest are shown clearly in Table I in which detailed data for three of the animals are presented. It will be observed that after oral administration of the benzoate, there was no significant amount of free (unconjugated) benzoic acid excreted even in the absence of supplementary glycine feeding. On the other hand, unconjugated benzoic acid in considerable amount was excreted after intravenous injection of the benzoate. These observations are in accord with those previously recorded (2), although since the dosage of benzoic acid per kilo is less than in earlier experiments, the excretion of free benzoic acid is smaller. Similarly, after intravenous injection of sodium phenylacetate the excretion of unconjugated phenylacetic acid was somewhat increased.

In previous studies (2), it was shown that maximal increases in the rate of hippuric acid excretion were obtained when 3 equivalents of glycine were fed and that administration of glycine in larger amounts did not significantly increase the synthesis and excretion of hippuric acid. Since the synthesis and excretion of phenaceturic acid took place so slowly even with the addition of glycine (3 equivalents) to the diet, it was of interest to determine whether the rate of excretion could be increased if a large amount of glycine were fed. The oral administration of 5 equivalents of glycine (Rabbit 14, Table I) did not result in any notably increased elimination of phenaceturic acid in the 6 hour period as compared with the experiments in which 3 equivalents were fed.

Since benzoic acid and phenylacetic acid may conjugate with glucuronic acid, the possibility is suggested that the excretion of total phenylacetic acid might not be as delayed as the excretion of phenaceturic acid and that the slow excretion of phenaceturic acid might be associated with an elimination of significant amounts of phenylacetic acid in combination with glucuronic acid or some substance other than glycine. An inspection of the values of Table I shows that the excretion of total phenylacetic acid runs parallel with the excretion of phenaceturic acid and that there is no considerable amount of conjugated phenylacetic acid other than that present as phenaceturic acid in the urine.

The rate of excretion of hippuric acid by the rabbit after its oral administration is practically identical with the rate of excretion of hippuric acid after benzoic acid is fed (7). In explanation of

this, Griffith (7, 8) has suggested that hippuric acid may be adsorbed from the intestine more slowly than benzoic acid, and that hippuric acid is hydrolyzed, in large part, to benzoic acid and glycine prior to absorption. Phenaceturic acid, in our own experiments, was excreted rapidly and completely after intravenous injection (Rabbit 31, Table I). On the other hand, when phenaceturic acid was fed (Rabbit 29, Table I), the excretion was greatly delayed, the amount eliminated in 6 hours being only slightly greater than the value obtained when phenylacetic acid and glycine were fed. While our limited supply of phenaceturic acid did not permit of further studies, the similarity of the results obtained after feeding hippuric and phenaceturic acids suggests that phenaceturic acid also may be hydrolyzed in the intestine prior to absorption.

After feeding benzoic acid with or without glycine, the excretion of the benzoic acid was practically completed within 24 hours after feeding,² the maximal excretion being obtained in the 6 hour period. The excretion of ingested phenylacetic acid was slow and was never complete within 24 hours. In a number of experiments, the excretion was prolonged over several days. When glycine was administered, the period required for approximately complete excretion was usually shortened. The most rapid elimination of the ingested phenylacetic acid was obtained with Rabbit 14, in which 90.5 per cent of the phenylacetic acid was excreted in the urine within 24 hours after feeding 1.839 gm. of phenylacetic acid and 5 equivalents of glycine. Typical data with two rabbits are presented in Table II.

Our results with rabbits are somewhat different from those of Quick (1) with dogs. He observed that when glycine was supplied exogenously, the speed of conjugation of glycine with phenylacetic acid was much faster than the conjugation with benzoic acid. In the dog both of these acids are conjugated with glu-

² Data concerning the completeness of the excretion of ingested benzoic acid in the 24 hour period immediately after its administration under the same experimental conditions as those of these experiments are presented in Table I of Paper VIII. Control experiments with benzoic acid were also carried out with the same animals as those of Table II, but since the experiments merely served as checks and are confirmatory of the other data, they are not reported in Table II.

curonic acid to a considerable extent. The addition of exogenous glycine (as gelatin, a protein rich in glycine) resulted in increases in the amount of phenaceturic acid excreted, while similar addi-

TABLE II

Rate of Elimination of Total "Extra" Phenylacetic Acid after Administration of Phenylacetic Acid Alone and with Glycine or Alanine

The amounts of organic acids administered per kilo of body weight were equivalent in every case. Except where indicated the acids were fed.

Rabbit No.	Period		Extra total phenylacetic acid		Amino acid fed		Rabbit No.	Period		Extra total phenylacetic acid		Amino acid fed	
	hrs.	gm.	per cent in-take		mols			hrs.	gm.	per cent in-take		mols	
29	6	0.356	20.2	Glycine	3	31	6	0.388	21.9	Glycine	3		
	18	0.789	44.7				18	0.782	44.3				
	Total	1.145	64.9				Total	1.170	66.2				
	24	0.432	24.5				24	0.470	26.6				
	6	0.114	6.5	Alanine	3		6	0.069	4.0	Alanine	3		
	18	0.187	10.6				18	0.375	21.2				
	Total	0.301	17.1				Total	0.444	25.2				
	24	0.395	22.4				24	0.470	26.6				
	24	0.567	32.1				24	0.516	29.2				
	24	0.295	16.7				24	0.177	10.0				
	6	0.158	9.0	0	6		0.126	7.1	0				
	18	0.330	18.7		18		0.670	38.0					
	Total	0.488	27.7		Total		0.796	45.1					
	24	0.498	28.2		24		0.489	27.7					
6	0.203	10.2*	Glycine	3	6	1.348	89.3†	0					
18	0.916	45.8			18	0.061	4.0						
Total	1.119	56.0			Total	1.409	93.3						
24	0.564	28.6											

* Intravenous injection.

† Phenaceturic acid equivalent to the phenylacetic acid was injected intravenously.

tion of gelatin to the diet increased the amount of urinary hippuric acid only slightly. With rabbits, the increases in the absolute amounts of hippuric acid excreted when glycine was fed were

much greater than in the case of phenaceturic acid. This serves to emphasize anew the differences between species in the ease and mode of conjugation of benzoic acid and its homologues.

Quick (1) observed no excretion of free (unconjugated) benzoic acid or phenylacetic acid by the dog after the administration of these acids. He therefore presented the hypothesis that compounds of this type introduced into the organism become "fixed" and that their "liberation" is brought about by conjugation. In the rabbit, we have observed the excretion of considerable amounts of these acids in unconjugated form after intravenous injection. The dosages per kilo of body weight used by us in these and in earlier studies with rabbits (2) were, however, somewhat larger than those of Quick in his studies with dogs. Further investigation of this hypothesis is desirable.

SUMMARY

1. When the sodium salt of phenylacetic acid was fed (0.736 gm. per kilo calculated as the acid) to rabbits, the excretion of phenaceturic acid was slow. When comparable amounts of benzoic and phenylacetic acids were fed, the average excretions of hippuric and phenaceturic acids in the 6 hour period immediately after feeding were equivalent to 37 and 6 per cent of the intake respectively.

2. If glycine was fed with the phenylacetic acid, the rate of excretion of phenaceturic acid was increased, but the average total excretion even under these favorable conditions was equivalent to only 21 per cent of the phenylacetic acid administered.

3. The slow excretion of phenaceturic acid was not associated with a delayed absorption of phenylacetic acid from the intestine, since when the phenylacetic acid was introduced directly into the blood stream, the rate of excretion of phenaceturic acid was not greater than after oral administration of the phenylacetic acid. Since after intravenous injection of phenaceturic acid, this acid is eliminated rapidly by the kidneys, it is believed that the slow excretion after the administration of phenylacetic acid was due to a limitation in the capacity to synthesize phenaceturic acid even though an exogenous supply of glycine was available.

4. The excretion of "extra" hippuric acid after administration of benzoic acid with or without exogenous glycine was usually

complete within 24 hours, while under similar conditions the excretion of "extra" phenaceturic acid was frequently prolonged for a period of several days after the administration of phenylacetic acid.

5. These experiments serve to emphasize the fact that even in the same species the two homologous acids, benzoic and phenylacetic, differ in their behavior in the living organism.

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